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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

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FILE 'SCISEARCH' ENTERED AT 17:05:16 ON 11 MAY 2004
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=> s termamyl-like alpha amylase and (mutant? or variant?)
L1          30 TERMAMYL-LIKE ALPHA AMYLASE AND (MUTANT? OR VARIANT?)

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          21 DUP REM L1 (9 DUPLICATES REMOVED)

=> s l2 and (H405 or H406)
L3           0 L2 AND (H405 OR H406)

=> s l2 and (405 or 406)
L4           0 L2 AND (405 OR 406)

=> s l2 and (405 or 407)
L5           0 L2 AND (405 OR 407)

=> d l2 1-21 ibib ab

L2   ANSWER 1 OF 21  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER:   2004:90380  BIOSIS
DOCUMENT NUMBER:    PREV200400094647
TITLE:              alpha-amylase mutants.
AUTHOR(S):          Borchert, Torben Vedel [Inventor, Reprint Author];
                    Svendsen, Allan [Inventor]; Andersen, Carsten [Inventor];
                    Nielsen, Bjarne [Inventor]; Nissen, Torben Lauesgaard
                    [Inventor]; Kjaerulff, Soren [Inventor]
CORPORATE SOURCE:   Copenhagen .O slashed., Denmark
                    ASSIGNEE: Novozymes A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6673589 January 06, 2004
SOURCE:             Official Gazette of the United States Patent and Trademark
                    Office Patents, (Jan 6 2004) Vol. 1278, No. 1.
                    http://www.uspto.gov/web/menu/patdata.html. e-file.
                    ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE:      Patent
LANGUAGE:           English
ENTRY DATE:         Entered STN: 11 Feb 2004
                    Last Updated on STN: 11 Feb 2004
AB   The invention relates to a variant of a parent Termamyl
     -like alpha -amylase, which exhibits an
     alteration in at least one of the following properties relative to said
     parent alpha -amylase: i) improved pH stability at a pH from 8 to 10.5;
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and/or ii) improved Ca2+ stability at pH 8 to 10.5, and/or iii) increased specific activity at temperatures from 10 to 60degree C.

L2 ANSWER 2 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:172894 BIOSIS
DOCUMENT NUMBER: PREV200300172894
TITLE: alpha-amylase **mutants**.
AUTHOR(S): Svendsen, Allan [Inventor, Reprint Author]; Borchert, Torben Vedel [Inventor]; Bisgard-Frantzen, Henrik [Inventor]; Outtrup, Helle [Inventor]; Nielsen, Bjarne Ronfeldt [Inventor]; Nielsen, Vibeke Skovgaard [Inventor]; Hedegaard, Lisbeth [Inventor]
CORPORATE SOURCE: Birkerod, Denmark
ASSIGNEE: Novozymes, A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6528298 March 04, 2003
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar 4 2003) Vol. 1268, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Apr 2003
Last Updated on STN: 2 Apr 2003

AB The invention relates to a novel **Termamyl-like alpha-amylase**, and Termamyl-like alpha-amylases comprising mutations in two, three, four, five or six regions/positions. The **variants** have increased thermostability at acidic pH and/or at low Ca2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an alpha-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an alpha-amylase **variant** of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an alpha-amylase **variant** of the invention, a manual or automatic dishwashing detergent composition comprising an alpha-amylase **variant** of the invention, a method for generating a **variant** of a parent **Termamyl-like alpha-amylase**, which **variant** exhibits increased thermostability at acidic pH and/or at low Ca2+ concentrations (relative to the parent).

L2 ANSWER 3 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:107515 HCAPLUS
DOCUMENT NUMBER: 136:163301
TITLE: Engineering and industrial use of **Termamyl-like .alpha.-amylase mutants** with increased stability at low pH, high temperature and low Ca2+ concentration
INVENTOR(S): Thisted, Thomas; Kjaerulff, Soren; Andersen, Carsten; Fuglsang, Claus Crone
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002010355	A2	20020207	WO 2001-DK488	20010712
WO 2002010355	A3	20030912		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1370648 A2 20031217 EP 2001-956424 20010712

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY, TR

JP 2004508815 T2 20040325 JP 2002-516073 20010712

US 2002155574 A1 20021024 US 2001-918543 20010731

PRIORITY APPLN. INFO.: DK 2000-1160 A 20000801
DK 2000-1354 A 20000912
DK 2000-1687 A 20001110
DK 2001-655 A 20010426
US 2000-225140P P 20000814
US 2000-233986P P 20000920
US 2000-249104P P 20001116
US 2001-286869P P 20010426
WO 2001-DK488 W 20010712

AB The present invention relates to **variants** (**mutants**) of parent Termamyl-like .alpha.-amylases, which **variant** has .alpha.-amylase activity and exhibits increased stability at low pH, high temp. and low Ca2+ concn. compared to the parent enzyme. The parent **Termamyl-like .alpha.-amylase** is derived from strains of Bacillus, B. licheniformis, B. amyloliquefaciens, and B. stearothermophilus. The **variants** of the invention are suitable for starch conversion, ethanol prodn., laundry wash, dish wash, hard surface cleaning, textile desizing, and/or sweetener prodn.

L2 ANSWER 4 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2002:236435 HCAPLUS

DOCUMENT NUMBER: 136:259230

TITLE: .alpha.-Amylases and .alpha.-amylase **variants** with improved properties for commercial uses

INVENTOR(S): Svendsen, Allan; Borchert, Torben Vedel; Bisgard-Frantzen, Henrik; Outtrup, Helle; Nielsen, Bjarne Ronfeldt; Nielsen, Vibeke Skovgaard; Hedegaard, Lisbeth

PATENT ASSIGNEE(S): Novozymes A/S, Den.

SOURCE: U.S., 64 pp., Cont.-in-part of U.S. 6,187,576.
CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6361989	B1	20020326	US 1999-290734	19990413
US 6187576	B1	20010213	US 1998-170670	19981013
WO 2000060060	A2	20001012	WO 2000-DK149	20000328
WO 2000060060	A3	20010419		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

BR 2000009392	A	20020108	BR 2000-9392	20000328
EP 1173554	A2	20020123	EP 2000-912416	20000328

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

JP 2002540786 T2 20021203 JP 2000-609552 20000328
US 6528298 B1 20030304 US 2000-545586 20000407
US 2003211958 A1 20031113 US 2002-327837 20021223

PRIORITY APPLN. INFO.: DK 1997-1172 A 19971013
US 1997-63306P P 19971028
US 1998-170670 A2 19981013
DK 1999-439 A 19990331
DK 1999-490 A 19990413
US 1999-290734 A 19990413
WO 2000-DK149 W 20000328
US 2000-545586 A3 20000407

AB The invention relates to a novel **Termamyl-like** . **alpha.-amylase**, and Termamyl-like .alpha.-amylases comprising mutations in two, three, four, five or six regions/positions. Specifically, **variants** are constructed by std. mol. biol. techniques with deletions of the I181 and G182 residues, and one or more of the substitutions N193F, L204F, E210H, and E214Q in BSG .alpha.-amylase. The **variants** have increased thermostability at acidic pH and/or at low Ca2+ concns. (relative to the parent). Genomic DNAs encoding novel .alpha.-amylases are also isolated from Bacillus strains DSM 12648 and DSM 12649. The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, or a cell which is transformed with a DNA construct of the invention. The use of .alpha.-amylase **variants** of the invention are useful for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive, a manual or automatic dishwashing detergent compn., or a method for generating a **variant** of a parent **Termamyl-like** **.alpha.-amylase** which **variant** exhibits increased thermostability at acidic pH and/or at low Ca2+ concns. (relative to the parent).

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:544951 BIOSIS
DOCUMENT NUMBER: PREV200200544951
TITLE: alpha-amylase **mutants**.
AUTHOR(S): Svendsen, Allan [Inventor, Reprint author]; Bisgard-Frantzen, Henrik [Inventor]; Borchert, Torben Vedel [Inventor]
CORPORATE SOURCE: Birkeroed, Denmark
ASSIGNEE: Novozymes A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6440716 August 27, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 27, 2002) Vol. 1261, No. 4. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Oct 2002
Last Updated on STN: 23 Oct 2002

AB The present invention relates to a method of constructing a **variant** of a parent **Termamyl-like** **alpha-amylase**, which **variant** has alpha-amylase activity and at least one altered property as compared to the parent alpha-amylase, comprises i) analysing the structure of the parent **Termamyl-like alpha-amylase** to identify at least one amino acid residue or at least one structural part of the **Termamyl-like alpha-amylase** structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent **Termamyl-**

like alpha-amylase (as evaluated on the basis of structural or functional considerations), ii) constructing a **Termamyl-like alpha-amylase variant**, which as compared to the parent **Termamyl-like alpha-amylase**, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and, optionally, iii) testing the resulting **Termamyl-like alpha-amylase variant** with respect to the property in question.

L2 ANSWER 6 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:423744 BIOSIS
DOCUMENT NUMBER: PREV200200423744
TITLE: Alpha-amylase **variants**.
AUTHOR(S): Andersen, Carsten [Inventor]; Jorgensen, Christel Thea [Inventor]; Bisgard-Frantzen, Henrik [Inventor]; Svendsen, Allan [Inventor]; Kjaerulff, Soren [Inventor]
CORPORATE SOURCE: ASSIGNEE: Novozymes A/S
PATENT INFORMATION: US 6410295 June 25, 2002
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (June 25, 2002) Vol. 1259, No. 4. http://www.uspto.gov/web/menu/patdata.html. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 7 Aug 2002
Last Updated on STN: 7 Aug 2002
AB The invention relates to a **variant** of a parent **Termamyl-like alpha-amylase**, which **variant** exhibits altered properties, in particular reduced capability of cleaving a substrate close to the branching point, and improved substrate specificity and/or improved specific activity relative to the parent alpha-amylase.

L2 ANSWER 7 OF 21 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-09677 BIOTECHDS
TITLE: Novel **variant** of parent **Termamyl-like alpha-amylase** useful for starch liquefaction, washing and/or dishwashing, has alpha-amylase activity and exhibits altered properties relative to the parent alpha-amylase;
vector-mediated gene transfer and expression in host cell for recombinant protein production
AUTHOR: SVENDSEN A; ANDERSEN C; THISTED T; VON DER OSTEN C
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2002092797 21 Nov 2002
APPLICATION INFO: WO 2002-DK319 15 May 2002
PRIORITY INFO: DK 2001-1443 2 Oct 2001; DK 2001-760 15 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-175077 [17]
AB DERWENT ABSTRACT:
NOVELTY - A **variant** (I) of parent **Termamyl-like alpha-amylase**, comprising an alteration at one or more positions (P) selected from 82 positions given in specification, where the alteration(s) are insertion, deletion or substitution of amino acid (a.a) which occupies (P), and each (P) corresponds to a position of the parent sequence comprising 483 a.as fully defined in the specification, is new.
DETAILED DESCRIPTION - A **variant** (I) of a parent **Termamyl-like alpha-amylase**, comprises an alteration at one or more positions (P) selected from 82 positions given in the specification such as 5, 6, 36 or 37, where the alteration(s) are independently an insertion of an amino acid (a.a) downstream of a.a which occupies (P), a deletion of a.a which occupies

(P) or a substitution of a.a which occupies (P) with a different a.a, where (I) has alpha-amylase activity, and each (P) corresponds to a position of the parent **Termamyl-like alpha-amylase** sequence (Bacillus licheniformis alpha-amylase) comprising 483 a.as fully defined in the specification. INDEPENDENT CLAIMS are also included for the following: (1) a DNA construct (II) comprising a DNA sequence encoding (I); (2) a recombinant expression vector (III) which carries (II); (3) a cell (IV) which is transformed with (II) or (III); and (4) a composition (V) comprising (I).

WIDER DISCLOSURE - Also disclosed is a detergent additive comprising (I).

BIOTECHNOLOGY - Preferred **Variant**: (I) comprises substitutions at approximately 1-483 amino acid positions, e.g., the e.g. amino acid at position 1 is substituted by A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y; the amino acid at position 2 is substituted by R,N,D,C,Q,E,G,H,I,L,K,M,F,S,T,W,Y,V; the amino acid at position 3 is substituted by A,R,N,D,C,Q,E,G,H,I,L,K,M,F,P,S,T,W,Y, all given in the specification. (I) comprises an alteration at one or more positions given in the specification such as A1 insertion, L3 insertion, L4 insertion, L7 insertion or deletion, etc. The parent **Termamyl-like alpha-amylase** is derived from a strain of B.licheniformis (comprising a sequence (S1) of 483 amino acids fully defined in the specification), B.amyloliquefaciens (comprising S1), B.stearothermophilus (comprising a sequence of 515 amino acids fully defined in the specification), Bacillus sp. (comprising a sequence (S2) of 485 amino acids fully defined in the specification (AAA560)), Bacillus sp. (comprising S2 (SP690)), Bacillus sp. (comprising S2 (SP722)), Bacillus sp. 707 alpha-amylase (comprising S2), KSM-AP1378. The parent **Termamyl-like alpha-amylase** has a sequence which has a degree of identity to S1 of at least 60% identity, preferably 99% identity. The parent **Termamyl-like alpha-amylase** is encoded by a nucleic acid sequence (DNA), which hybridizes under low, preferably medium, more preferably high stringency conditions, with a sequence of 1920 base pairs fully defined in the specification. Preferred Cell: (V) is a microorganism, preferably a fungus or bacterium selected from B.subtilis, B.licheniformis, B.lentus, B.brevis, B.stearothermophilus, B.alkalophilus, B.amyloliquefaciens, B.coagulans, B.circulans, B.lactus and B.thuringiensis. Preferred Composition: (V) further comprises glucoamylase, pullulanase and/or phytase. (V) is preferably a detergent composition and additionally comprises another enzyme such as a protease, lipase, peroxidase, another amylolytic enzyme, glucoamylase, maltogenic amylase, CGTase, mannanase, cutinase, laccase and/or cellulase.

USE - (I) or (V) is useful for starch liquefaction, in particular for syrup or ethanol production, for washing and/or dishwashing, or for textile desizing (claimed). (I) is useful for desizing fabrics and garments, in beer making or brewing, and in pulp and paper production.

ADVANTAGE - (I) has alpha-amylase activity and exhibits an alteration in at least one of the following properties relative to the parent alpha-amylase: altered substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH activity profile, pH stability profile, stability towards oxidation, Ca2+ dependency, reduced and increased pH and improved wash performance, specific activity, stability under high temperature and/or low pH conditions, in particular at low calcium concentrations and/or in particular at high temperatures from 70-120degreesC and/or low pH in the range from pH 4-6 (claimed). (I) has reduced sensitivity (or improves stability against denaturation) to anionic surfactants.

EXAMPLE - A **variant** of parent **Termamyl-like alpha-amylase** was constructed as described in

EXAMPLE 1 of WO20029560 in the parent Bacillus licheniformis approximately a-amylase having a sequence of 483 amino acids fully defined in the specification. (84 pages)

L2 ANSWER 8 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2001:676914 HCAPLUS
DOCUMENT NUMBER: 135:238614
TITLE: Tertiary structure modeling of Bacillus .alpha.-amylases and construction of **variants** with altered solubility and related enzymic properties
INVENTOR(S): Andersen, Carsten; Borchert, Torben Vedel; Nielsen, Bjarne Ronfeldt
PATENT ASSIGNEE(S): Novozymes A/S, Den.
SOURCE: PCT Int. Appl., 152 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001066712	A2	20010913	WO 2001-DK144	20010307
WO 2001066712	A3	20020418		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1263942	A2	20021211	EP 2001-911458	20010307
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004505606	T2	20040226	JP 2001-565869	20010307
US 2003129718	A1	20030710	US 2001-925576	20010809

PRIORITY APPLN. INFO.:

DK 2000-376	A	20000308
US 2000-189857P	P	20000315
DK 2001-303	A	20010223
US 2001-271382P	P	20010226
WO 2001-DK144	W	20010307

AB The present invention relates to **variants** (**mutants**) of polypeptides, in particular Termamyl-like .alpha.-amylases, which **variant** has .alpha.-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent .alpha.-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidn., Ca2+ dependency, specific activity, and soly. Thus, crystals of the alk. **Termamyl-like** . **alpha.-amylase** (SP722) are obtained by the hanging drop method, and the at. coordinates and tertiary structure of SP722 provided. Two interaction zones surrounding the active site interact with the same two areas on an antiparallel neighbor mol.; likewise, the backside zone is in contact with the backside zone on a third antiparallel amylase mol., although all constacts here are water mediated. Amino acid residues being less than 6.0 or 3.5 .ANG. from the nearest neighboring amylase mol. are identified in the model structure of SP722 amylase. A model of another alk. Termamyl-like amylase, AA560, is built based on the SP722 tertiary structure. Localized random, doped mutagenesis of AA560 .alpha.-amylase yields **variants** having increased soly. in comparison to the parent enzyme. In particular, the .DELTA.(D183-D184)+N195F+N445Q+K446N **variant** of AA450 has soly. of >6 mg/mL in comparison to 2 mg/mL for wild-type AA450. The genes encoding wild-type and **variant** AA560 are located in plasmid pTVB223 and expressed from the amyL promoter in Bacillus subtilis. Such **variants** have useful com. applications, such as in dishwashing and laundry detergents, textile desizing, and starch liquefaction.

L2 ANSWER 9 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 2001:161441 HCAPLUS
DOCUMENT NUMBER: 134:190018
TITLE: .alpha.-Amylase **variants** with improved detergent performance
INVENTOR(S): Svendsen, Allan; Kjaerulff, Soeren; Bisgaard-Frantzen, Henrik; Andersen, Carsten
PATENT ASSIGNEE(S): Novo-Nordisk A/S, Den.; Novo Alle
SOURCE: U.S., 36 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6197565	B1	20010306	US 1998-193068	19981116

PRIORITY APPLN. INFO.: US 1998-193068 19981116

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, comprising mutations in two, three, four, five or six regions/positions. The **variants** have increased stability at high temps. (relative to the parent). The **variants** comprise addnl. mutations added to the LE174 hybrid .alpha.-enzyme in which the 35 N-terminal residues of Bacillus licheniformis .alpha.-amylase are replaced by residues 1-33 of BAN/B. amyloliquefaciens .alpha.-amylase. The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase **variant** of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase **variant** of the invention, a manual or automatic dishwashing detergent compn. comprising an .alpha.-amylase **variant** of the invention, a method for generating a **variant** of a parent **Termamyl-like .alpha.-amylase**, which **variant** exhibits increased.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 10 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:427475 BIOSIS
DOCUMENT NUMBER: PREV200100427475
TITLE: alpha-amlase **mutants**.
AUTHOR(S): Borchert, Torben Vedel [Inventor, Reprint author]; Svendsen, Allan [Inventor]; Andersen, Carsten [Inventor]; Nielsen, Bjarne [Inventor]; Nissen, Torben Lauesgaard [Inventor]; Kjaerulff, Soren [Inventor]
CORPORATE SOURCE: Copenhagen, Denmark
ASSIGNEE: Novo Nordisk A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6204232 March 20, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 20, 2001) Vol. 1244, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Sep 2001
Last Updated on STN: 22 Feb 2002

AB The invention relates to a **variant** of a parent **Termamyl -like alpha-amylase**, which exhibits an alteration in at least one of the following properties relative to said parent alpha-amylase: i) improved pH stability at a pH from 8 to 10.5; and/or ii) improved Ca2+ stability at pH 8 to 10.5, and/or iii) increased

specific activity at temperatures from 10 to 60degree C.

L2 ANSWER 11 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:354951 BIOSIS
DOCUMENT NUMBER: PREV200100354951
TITLE: alpha-amylase **mutants**.
AUTHOR(S): Svendsen, Allan [Inventor, Reprint author]; Borchert, Torben Vedel [Inventor]; Bisgard-Frantzen, Henrik [Inventor]
CORPORATE SOURCE: Birkerod, Denmark
ASSIGNEE: Novo Nordisk A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6187576 February 13, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 13, 2001) Vol. 1243, No. 2. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002

AB The invention relates to a **variant** of a parent **Termamyl -like alpha-amylase**, comprising mutations in two, three, four, five or six regions/positions. The **variants** have increased thermostability at acidic pH and/or at low Ca2+ concentrations (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an alpha-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an alpha-amylase **variant** of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an alpha-amylase **variant** of the invention, a manual or automatic dishwashing detergent composition comprising an alpha-amylase **variant** of the invention, a method for generating a **variant** of a parent **Termamyl-like alpha-amylase**, which **variant** exhibits increased thermostability at acidic pH and/or at low Ca2+ concentrations (relative to the parent).

L2 ANSWER 12 OF 21 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-07723 BIOTECHDS
TITLE: New **variant** of parent **Termamyl-like alpha-amylase** for use as a component in washing and dishwashing compositions, for textile desizing, for starch liquefaction, and for producing sweeteners and ethanols from starch; recombinant vector-mediated gene transfer and expression in fungus or bacterium cell for use in starch liquefaction and surfactant, ethanol and sweetener preparation
AUTHOR: SVENDSEN A; JORGENSEN C T; NIELSEN B R
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2001088107 22 Nov 2001
APPLICATION INFO: WO 2000-DK323 12 May 2000
PRIORITY INFO: DK 2000-779 12 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-106123 [14]

AB DERWENT ABSTRACT:
NOVELTY - A **variant** (I) of parent **Termamyl-like alpha-amylase** comprising an alteration at regions 186-193, 261-276, 283-293 or 334-339, or at position 234, where (I) has alpha-amylase activity and each position corresponds to a position of a parent **Termamyl-like alpha-amylase** sequence having a Bacillus licheniformis alpha-amylase sequence of 483 amino acids, given in specification, is new.
DETAILED DESCRIPTION - A new **variant** (I) of parent

Termamyl-like alpha-amylase comprises an alteration at regions 186-193, 261-276, 283-293 or 334-339, or at position 234, where (I) has alpha-amylase activity and each position corresponds to a position of a parent **Termamyl-like alpha-amylase** sequence having a Bacillus licheniformis alpha-amylase sequence of 483 amino acids, given in specification. The alteration(s) are independently: (a) an insertion of an amino acid downstream of the amino acid which occupies the position; (b) deletion of the amino acid which occupies the position; or (c) substitution of the amino acid which occupies the position with a different amino acid. INDEPENDENT CLAIMS are also included for the following: (1) a DNA construct (II) comprising a DNA sequence encoding (I); (2) a recombinant expression vector (III) which carries (II); (3) a cell (IV) which is transformed with (II) or (III); (4) a detergent additive (V) comprising (I), optionally in the form of a non-dusting granulate, stabilized liquid or protected enzyme; (5) a detergent composition (VI) comprising (I); (6) a manual or automatic dishwashing detergent composition or laundry washing composition (VII) comprising (I); and (7) a composition (VIII) comprising (I).

BIOTECHNOLOGY - Preparation: Producing (I) involves cultivating a host cell under conditions conducive to the production of (I) and recovering (I) from the cells and/or culture medium. Preferred Mutation: (I) has a mutation at a position such as Trp263, Glu189, Lys335, Tyr290, Asn265, Val286, Gln264 or Lys234. (I) has mutations such as: (i) Trp263Gly Ala Ser Thr Val; (ii) Glu189Gly Ala Ser Thr Val; (iii) Leu335Gly Ala Ser Thr Val; (iv) Tyr290Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Val; (v) Asn265Gly, Ala, Ser, Thr, Val; (vi) Val286Phe, Trp, Tyr, Gly, Ala, Ser; (vii) Gln264X, Lys234X, preferably Asn Gln. The parent **Termamyl-like alpha-amylase** is derived from a strain of B. licheniformis, B. amyloliquefaciens, B. stearothermophilus, Bacillus sp. National Collection of Industrial Bacteria (NCIB) 12289, NCIB 12412, NCIB 12513 or DSM9375 or DSMZ no.12649, KSM AP1378. The parent Termamyl-like alpha-amylases is selected from a sequence of 485, 515, or 483 amino acids, given in the specification, or a sequence which has a degree of identity to the 485 base pair sequence of 60 %, preferably 99 %. The parent **Termamyl-like alpha-amylase** is encoded by a nucleic acid sequence, which hybridizes under low, preferably medium, more preferably high stringency conditions, with a sequence comprising 1920 base pairs, given in the specification. Preferred Cell: (IV) is a microorganism, preferably a fungus or a bacterium such as B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. coagulans, B. circulans, B. lautus or B. thuringiensis. Preferred Composition: (V) contains 0.02 - 200 mg of enzyme protein/g of (V). (V), (VI) or (VII) additionally comprises another enzyme such as a protease, lipase, peroxidase, amylase or another amylolytic enzyme, such as glucoamylase, and/or cellulase. (VIII) further comprises another alpha-amylase, glucoamylase, pullulanase, isoamylase, protease, preferably acidic protease, especially from Aspergillus, such as A. niger or a A. aculatus.

USE - (I), a detergent additive (V) comprising (I), a detergent composition (VI) comprising (I), or a composition (VIII) comprising (I) is useful for washing and/or dishwashing or textile desizing. (I) or (VIII) is useful for starch liquefaction or ethanol production (claimed). (I) is useful as a component in a hard surface cleaning detergent composition, and for producing sweeteners from starch.

ADVANTAGE - (I) has altered alpha-1, 6-D-glucosidic branch linkage cleavage activity on amylopectin, preferably increased alpha-1, 6-D-glucosidic branch linkage cleavage activity of amylopectin or a limit dextrin prepared by TERMAMYL (RTM) or NOVAMYL (RTM) (claimed).

EXAMPLE - **Variants** of parent **Termamyl-like alpha-amylase** such as: (i) Trp263GAla, Ser, Thr, Val; (ii) Asn265Gly, Ala, Ser, Thr, Val; (iii) Val286Phe, Trp, Tyr, Gly, Ala, Ser; (iv) Tyr290Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Val; (v) Leu335Gly, Ala, Ser,

Thr, Val; and (vi) Lys234Asn, Gln were constructed, as in EXAMPLE 1 of WO 00/29560 (from Novozymes A/S) in the Bacillus licheniformis alpha-amylase. The altered 1,6-activity was determined as follows. The enzyme solutions (of a chosen activity, e.g., 10-100 NU) were diluted with D2O and freeze-dried. The samples were re-dissolved in D2O (0.5 mL) and freeze-dried. Samples containing 25 mg of substrate in D2O (0.5 mL) were freeze-dried before re-dissolving (D2O 0.5 mL) and freeze-dried. Finally the enzymes were dissolved in D2O (1 mL) and added to each sample of substrate. The solutions were transferred to nuclear magnetic resonance (NMR) tubes and incubated at 60 degreesC. 1H NMR spectra were recorded currently at 60 degreesC on a Varian Mercury 400 MHz instrument. (84 pages)

L2 ANSWER 13 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 2000:725751 HCAPLUS
DOCUMENT NUMBER: 133:292888
TITLE: .alpha.-Amylase **variants** with improved specificity and/or specific activity
INVENTOR(S): Andersen, Carsten; Jorgensen, Christel Thea; Bisgard-Frantzen, Henrik; Svendsen, Allan; Kjaerulff, Soren
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 78 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000060059	A2	20001012	WO 2000-DK148	20000328
WO 2000060059	A3	20010510		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
BR 2000009362	A	20011226	BR 2000-9362	20000328
EP 1165762	A2	20020102	EP 2000-912415	20000328
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002540785	T2	20021203	JP 2000-609551	20000328
US 6410295	B1	20020625	US 2000-537168	20000329
US 2003044954	A1	20030306	US 2002-146327	20020515

PRIORITY APPLN. INFO.:

DK 1999-437	A	19990330
US 1999-127427P	P	19990401
WO 2000-DK148	W	20000328
US 2000-537168	A3	20000329

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, which **variant** exhibits altered properties, in particular reduced capability of cleaving a substrate close to the branching point, and improved substrate specificity and/or improved specific activity relative to the parent .alpha.-amylase. Thus, **variants** of Bacillus licheniformis are prepd. comprising various amino acid substitutions as well as substitution of the 35 N-terminal residues substituted by the 33 N-terminal residues of B. amyloliquefaciens .alpha.-amylase. The .alpha.-amylase **variants** have uses for starch liquefaction, laundry or dishwashing detergents, hard surface cleaning compns., ethanol prodn. for fuel or drinking, and desizing of textiles or fabrics.

L2 ANSWER 14 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 2000:351648 HCAPLUS
DOCUMENT NUMBER: 133:14086
TITLE: Bacillus **Termamyl-like** .
alpha.-amylase variants
with improved pH and temperature stability
INVENTOR(S): Svendsen, Allan; Kjaerulff, Soren; Bisgard-Frantzen, Henrik; Andersen, Carsten
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 80 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029560	A1	20000525	WO 1999-DK628	19991116
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1131418	A1	20010912	EP 1999-972255	19991116
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 2002530072	T2	20020917	JP 2000-582544	19991116

PRIORITY APPLN. INFO.: DK 1998-1495 A 19981116
WO 1999-DK628 W 19991116

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, comprising mutations in two, three, four, five or six regions/positions. The **variants** have increased stability at high temps. (relative to the parent). Thus, a triple mutation (L176R+I201F+W205N) was introduced into a hybrid .alpha.-amylase comprising residues 1-33 of Bacillus amyloliquefaciens .alpha.-amylase fused to residues 36-483 of B. licheniformis .alpha.-amylase. This construct has improved stability at high pH and temp. The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, and a cell which is transformed with a DNA construct of the invention. The .alpha.-amylase **variants** of the invention can be used for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase **variant** of the invention, or a manual or automatic dishwashing detergent compn.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 15 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:257063 BIOSIS
DOCUMENT NUMBER: PREV200100257063
TITLE: alpha-amylase **mutants**.
AUTHOR(S): Svendsen, Allan [Inventor, Reprint author]; Borchert, Torben Vedel [Inventor]; Bisgard-Frantzen, Henrik [Inventor]
CORPORATE SOURCE: Birkerod, Denmark
ASSIGNEE: Novo Nordisk A/S, Bagsvaerd, Denmark
PATENT INFORMATION: US 6143708 November 07, 2000

SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 7, 2000) Vol. 1240, No. 1. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 30 May 2001
Last Updated on STN: 19 Feb 2002

AB The invention relates to a **variant** of a parent **Termamyl -like alpha-amylase**, which **variant** has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca2+ dependency and specific activity.

L2 ANSWER 16 OF 21 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:345689 BIOSIS
DOCUMENT NUMBER: PREV200000345689
TITLE: alpha-amylase **mutants**.
AUTHOR(S): Svendsen, Allan [Inventor, Reprint author]; Bisg[ang]rd-Frantzen, Henrik [Inventor]; Borchert, Torben [Inventor]
CORPORATE SOURCE: Birkeroed, Denmark
ASSIGNEE: Novo Nordisk A/S, Bagsv.ae butted.rd, Denmark
PATENT INFORMATION: US 6022724 February 08, 2000
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 8, 2000) Vol. 1231, No. 2. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Aug 2000
Last Updated on STN: 7 Jan 2002

AB The present invention relates to a method of constructing a **variant** of a parent **Termamyl-like alpha-amylase**, which **variant** has alpha-amylase activity and at least one altered property as compared to the parent alpha-amylase, comprises i) analyzing the structure of the parent **Termamyl-like alpha-amylase** to identify at least one amino acid residue or at least one structural part of the **Termamyl-like alpha-amylase** structure, which amino acid residue or structural part is believed to be of relevance for altering the property of the parent **Termamyl-like alpha-amylase** (as evaluated on the basis of structural or functional considerations), ii) constructing a **Termamyl-like alpha-amylase variant**, which as compared to the parent **Termamyl-like alpha-amylase**, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and, optionally, iii) testing the resulting **Termamyl-like alpha-amylase variant** with respect to the property in question.

L2 ANSWER 17 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 1999:311291 HCAPLUS
DOCUMENT NUMBER: 130:334680
TITLE: .alpha.-Amylase **mutants** with improved wash performance
INVENTOR(S): Borchert, Torben Vedel; Svendsen, Allan; Andersen, Carsten; Nielsen, Bjarne Ronfeld; Nissen, Torben Lauesgaard; Kjaerulff, Soren
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9923211	A1	19990514	WO 1998-DK471	19981030
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2308119	AA	19990514	CA 1998-2308119	19981030
AU 9897373	A1	19990524	AU 1998-97373	19981030
EP 1027428	A1	20000816	EP 1998-951291	19981030
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
BR 9813328	A	20000822	BR 1998-13328	19981030
US 6204232	B1	20010320	US 1998-183412	19981030
JP 2001521739	T2	20011113	JP 2000-519071	19981030
US 2001039253	A1	20011108	US 2001-769864	20010125
US 6673589	B2	20040106		
US 2004038368	A1	20040226	US 2003-665667	20030919

PRIORITY APPLN. INFO.:

DK 1997-1240	A	19971030
DK 1998-936	A	19980714
US 1997-64662P	P	19971106
US 1998-93234P	P	19980717
US 1998-183412	A3	19981030
WO 1998-DK471	W	19981030
US 2001-769864	A3	20010125

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, which exhibits an alteration in at least one of the following properties relative to said parent .alpha.-amylase: (i) improved pH stability at a pH from 8 to 10.5; and/or (ii) improved Ca2+ stability at pH 8 to 10.5, and/or (iii) increased specific activity at temps. from 10 to 60.degree.. Thus, **variants** were prepd. from wild-type .alpha.-amylases from Bacillus strain NCIB 12512, Kasamyl (Bacillus strain NCIB 12513), Termamyl (Bacillus licheniformis), and B. amyloliquefaciens.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 18 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 7
ACCESSION NUMBER: 1999:271480 HCAPLUS
DOCUMENT NUMBER: 130:308445
TITLE: .alpha.-Amylase **mutants** with improved thermostability for use as detergent additives and for starch liquefaction
INVENTOR(S): Svendsen, Allan; Borchert, Torben Vedel; Bisgard-Frantzen, Henrik
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 93 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9919467	A1	19990422	WO 1998-DK444	19981013
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW,				

MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES,
FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI,
CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

CA 2305191	AA	19990422	CA 1998-2305191	19981013	
AU 9894343	A1	19990503	AU 1998-94343	19981013	
EP 1023439	A1	20000802	EP 1998-947417	19981013	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI					
JP 2001520006	T2	20011030	JP 2000-516020	19981013	

PRIORITY APPLN. INFO.: DK 1997-1172 A 19971013
WO 1998-DK444 W 19981013

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, comprising mutations in two, three, four, five or six regions/positions. The **variants** have increased thermostability at acidic pH and/or at low Ca2+ concns. (relative to the parent). The invention also relates to a DNA construct comprising a DNA sequence encoding an .alpha.-amylase **variant** of the invention, a recombinant expression vector which carries a DNA construct of the invention, a cell which is transformed with a DNA construct of the invention, the use of an .alpha.-amylase **variant** of the invention for washing and/or dishwashing, textile desizing, starch liquefaction, a detergent additive comprising an .alpha.-amylase **variant** of the invention, a manual or automatic dishwashing detergent compn. comprising an .alpha.-amylase **variant** of the invention, a method for generating a **variant** of a parent **Termamyl-like .alpha.-amylase**, which **variant** exhibits increased thermostability at acidic pH and/or at low Ca2+ concns. (relative to the parent). Preferred **variants** comprise: (1) the Bacillus stearothermophilus .alpha.-amylase wild-type sequence in which residues Ile181 and Gly182 are deleted and Asn193 is substituted by Phe (designated as the TVB146 **variant**), and (2) a hybrid **variant** comprising the 445 C-terminal residues of B. licheniformis .alpha.-amylase linked to the 37 N-terminal residues of B. amyloliquefaciens .alpha.-amylase plus the substitutions H156Y+A181T+N190F+A209V+Q264S (B. licheniformis numbering) (designated as the LE174 **variant**).

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 19 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:595405 HCAPLUS
DOCUMENT NUMBER: 131:198844
TITLE: Enzymatic preparation of glucose syrup from starch
INVENTOR(S): Norman, Barrie Edmund; Hendriksen, Hanne Vang
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 36 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9946399	A1	19990916	WO 1999-DK114	19990308
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2323068	AA	19990916	CA 1999-2323068	19990308

AU 9926124	A1	19990927	AU 1999-26124	19990308	
EP 1062359	A1	20001227	EP 1999-906094	19990308	
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI					
US 6287826	B1	20010911	US 1999-264097	19990308	
JP 2002505885	T2	20020226	JP 2000-535766	19990308	
PRIORITY APPLN. INFO.:			DK 1998-321	A	19980309
			US 1998-79209P	P	19980324
			WO 1999-DK114	W	19990308

AB The present invention relates to a process for the prepn. of a glucose syrup wherein starch is treated with a **Termamyl-like** . **alpha**.-**amylase** comprising a substitution in Val54 shown in SEQ ID NO: 2 or in the corresponding position in another **Termamyl-like** .**alpha**.-**amylase**. The invention also relates to a glucose syrup obtainable by the process of the invention and the use thereof as ingredient in food products. An object of the invention is also to provide for the use of a **Termamyl-like** .**alpha**.-**amylase** with a substitution in position Val54 using SEQ ID NO: 2 as the backbone or a corresponding position in another **Termamyl-like** .**alpha**.-**amylase** for prepg. glucose syrup. A glucose syrup was prepd. by treating a starch slurry contg. 30 % dry solid waxy maize starch, 40 ppm Ca2+ at pH 6 with 0.1 mg enzyme protein/g dry solid of Val54Trp substituted Bacillus licheniformis .alpha.-amylase. The temp. was kept at 95.degree. for 1 h and 80.degree. for 72 h. The sugar spectrum of the obtained glucose syrup was compared with the spectrum of 42 DE (dextrose equiv.) acid converted syrup.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 20 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 8

ACCESSION NUMBER:	1997:740294 HCAPLUS
DOCUMENT NUMBER:	128:20052
TITLE:	Recombinant alpha-amylase **mutants** and their use in textile desizing, starch liquefaction and washing
INVENTOR(S):	Svendsen, Allan; Borchert, Torben Vedel; Bisgard-Frantzen, Henrik
PATENT ASSIGNEE(S):	Novo Nordisk A/S, Den.; Svendsen, Allan; Borchert, Torben Vedel; Bisgard-Frantzen, Henrik
SOURCE:	PCT Int. Appl., 100 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1
PATENT INFORMATION:	

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9741213	A1	19971106	WO 1997-DK197	19970430
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9726928	A1	19971119	AU 1997-26928	19970430
EP 904360	A1	19990331	EP 1997-920604	19970430
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
CN 1217020	A	19990519	CN 1997-194297	19970430
BR 9708887	A	19990803	BR 1997-8887	19970430
JP 2000508914	T2	20000718	JP 1997-538373	19970430
US 6143708	A	20001107	US 1998-182859	19981029
US 6436888	B1	20020820	US 2000-672459	20000928

US 2003171236	A1	20030911	US 2002-186042	20020628		
US 6642044	B2	20031104				
US 2004048351	A1	20040311	US 2003-644187	20030820		
PRIORITY APPLN. INFO.:			DK 1996-515	A	19960430	
			DK 1996-712	A	19960628	
			DK 1996-775	A	19960711	
			DK 1996-1263	A	19961108	
			WO 1997-DK197	W	19970430	
			US 1998-182859	A1	19981029	
			US 2000-672459	A3	20000928	
			US 2002-186042	A3	20020628	

AB The invention relates to a **variant** of a parent **Termamyl -like .alpha.-amylase**, which **variant** has .alpha.-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidn., Ca2+ dependency and specific activity. Many Bacillus licheniformis .alpha.-amylase **variants** altered in thermal stability, pH stability, Ca2+ dependency and specific activity were prepd. Improved starch liquefaction with with these enzymes was demonstrated.

L2 ANSWER 21 OF 21 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1996:584142 HCAPLUS
DOCUMENT NUMBER: 125:241792
TITLE: A method of designing alpha-amylase **mutants** with predetermined properties, alpha-amylase **variants**, and detergents containing the **variants**
INVENTOR(S): Svendsen, Allan; Bisgaard-Frantzen, Henrik; Borchert, Torben Vedel
PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.
SOURCE: PCT Int. Appl., 171 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9623874	A1	19960808	WO 1996-DK57	19960205
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE				
CA 2211316	AA	19960808	CA 1996-2211316	19960205
AU 9644834	A1	19960821	AU 1996-44834	19960205
BR 9607013	A	19971028	BR 1996-7013	19960205
EP 808363	A1	19971126	EP 1996-900895	19960205
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE				
CN 1172501	A	19980204	CN 1996-191745	19960205
JP 11500003	T2	19990106	JP 1996-523187	19960205
US 5989169	A	19991123	US 1996-600908	19960213
US 6022724	A	20000208	US 1996-683838	19960718
US 6440716	B1	20020827	US 2000-636252	20000810
US 2003170769	A1	20030911	US 2002-184771	20020628
PRIORITY APPLN. INFO.:			DK 1995-128	A 19950203
			DK 1995-1192	A 19951023
			DK 1995-1256	A 19951110
			WO 1996-DK57	W 19960205
			US 1996-600908	A2 19960213
			US 1996-683838	A1 19960718

US 1999-327563 A1 19990608
US 2000-636252 A1 20000810

AB A method of constructing a **variant** of a parent **Termamyl -like .alpha.-amylase**, which **variant** has .alpha.-amylase activity and at least one altered property as compared to the parent .alpha.-amylase, comprises i) analyzing the structure of the parent **Termamyl-like .alpha.-amylase** to identify at least one amino acid residue or at least one structural part of the **Termamyl-like .alpha.-amylase** (as evaluated on the basis of structural or functional considerations), ii) constructing a **Termamyl-like .alpha.-amylase variant**, which as compared to the parent **Termamyl-like .alpha.-amylase**, has been modified in the amino acid residue or structural part identified in i) so as to alter the property, and iii) testing the resulting **Termamyl-like .alpha.-amylase variant** for the property in question. The resulting Termamyl **variants** and detergents contg. the **variants** are claimed. [Trp-54]- and [Trp-52,Trp-54]-Termamyl **variants** were prepd. with recombinant Bacillus subtilis. Model building had identified these residues as being important for substrate specificity. Alteration of these residues altered the substrate specificity to be more like that of Fungamyl (Aspergillus oryzae .alpha.-amylase).

=> d his

(FILE 'HOME' ENTERED AT 17:04:31 ON 11 MAY 2004)

FILE 'HCAPLUS, EMBASE, MEDLINE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 17:05:16 ON 11 MAY 2004

L1 30 S TERMAMYL-LIKE ALPHA AMYLASE AND (MUTANT? OR VARIANT?)
L2 21 DUP REM L1 (9 DUPLICATES REMOVED)
L3 0 S L2 AND (H405 OR H406)
L4 0 S L2 AND (405 OR 406)
L5 0 S L2 AND (405 OR 407)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	57.15	57.36
DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-7.62	-7.62

STN INTERNATIONAL LOGOFF AT 17:09:04 ON 11 MAY 2004

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Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6642044 B2

L1: Entry 1 of 1 File: USPT Nov 4, 2003

US-PAT-NO: 6642044
DOCUMENT-IDENTIFIER: US 6642044 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisgard-Frantzen; Henrik	Bagsvaerd			DK

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Novozymes A/S	Bagsvaerd			DK	03

APPL-NO: 10/ 186042 [PALM]
DATE FILED: June 28, 2002

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a division of 09/672,459, filed on Sep. 28, 2000 (now U.S. Pat. No. 6,436,888), which is a continuation of 09/182,859, filed on Oct. 29, 1998 (now U.S. Pat. No. 6,143,708), which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0515/96	April 30, 1996
DK	0712/96	June 28, 1996
DK	0775/96	July 11, 1996
DK	1263/96	November 8, 1996

INT-CL: [07] C12 N 1/20, C12 N 15/00, C12 N 9/28, C07 H 21/04

US-CL-ISSUED: 435/252.3; 435/202, 435/320.1, 536/23.2, 536/23.7, 510/226
US-CL-CURRENT: 435/252.3; 435/202, 435/320.1, 510/226, 536/23.2, 536/23.7

FIELD-OF-SEARCH: 435/252.3, 435/320.1, 435/202, 536/23.2, 536/23.7, 510/226

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
5731280	March 1998	Nielsen et al.	510/392
5736499	April 1998	Mitchinson et al.	510/392
5824532	October 1998	Barnett et al.	435/202

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 91/00353	January 1991	WO	
WO 95/10603	April 1995	WO	
WO 95/35382	December 1995	WO	
WO 96/23874	August 1996	WO	

ART-UNIT: 1652

PRIMARY-EXAMINER: Saidha; Tekchand

ATTY-AGENT-FIRM: Lambiris; Elias J. Garbell; Jason I.

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to said parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

6 Claims, 9 Drawing figures

Terms	Documents
6,642,044	1

Display Format: CIT Change Format

Previous Page Next Page Go to Doc#

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L2: Entry 2 of 2 File: USPT Aug 20, 2002

US-PAT-NO: 6436888
DOCUMENT-IDENTIFIER: US 6436888 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd			DK

US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

CLAIMS:

What is claimed is:

1. A variant of a parent Termamyl-like .alpha.-amylase, wherein said variant has .alpha.-amylase activity and exhibits an alteration relative to said parent .alpha.-amylase in at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH-activity profile, pH-stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity; said variant comprising at least two substitutions at positions corresponding to positions in the amino acid sequence of SEQ ID NO:2 selected from the group consisting of: H68, H247, H382, H450, N172, N188; N190, A209, A210, Q264, and N265.

2. A variant as defined in claim 1, further comprising at least one substitution at a position corresponding to a position in the amino acid sequence of SEQ ID NO:2 selected from the group consisting of: H133, H156, A181, G310, H450, V128, N104, V54, S187,H293, and A294.

3. A variant as defined in claim 1, further comprising at least one mutation selected from the group consisting of: V54L,I F,Y,W,R,K,H,E,Q; D53L,I,F,Y,W; Y56W; Q333W; G57A,R,D,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V; A52W,Y,L,F,I.

4. A variant as defined in claim 1, further comprising a substitution at a position corresponding to 1201 in SEQ ID NO:2.

5. A variant as defined in claim 1, wherein the parent Termamyl-like .alpha.-amylase is selected from the group consisting of: the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID No. 2, the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID

No. 4, the B. stearothennophilus .alpha.-amylase having the sequence shown in SEQ ID No. 6, the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in FIG. 1 and 2, the Bacillus strain NCIB 12513 .alpha.-amylase having the sequence shown in FIG. 2, and the Bacillus sp. #707 .alpha.-amylase having the sequence shown in FIG. 2.

6. A variant as defined in claim 1, wherein the parent .alpha.-amylase is B. stearothermophilus .alpha.-amylase SEQ ID NO:6 and the variant further pairwise deletions selected from the group consisting of R179*+G180* and I181*+G182*(using the numbering of SEQ ID No. 6).

7. A detergent additive comprising an .alpha.-amylase variant according to claim 1.

8. A detergent additive as defined in claim 7, which contains 0.02-200 mg of .alpha.-amylase protein/g of the additive.

9. A detergent additive as defined in claim 7, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

10. A detergent composition comprising an .alpha.-amylase variant according to claim 1.

11. A detergent composition as defined in claim 10, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

12. A manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant as defined in claim 1.

13. A dishwashing detergent composition as defined in claim 12, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

14. A manual or automatic laundry washing composition comprising an .alpha.-amylase variant as defined in claim 1.

15. A laundry washing composition as defined in claim 14, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, an amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

16. A composition comprising a mixture of .alpha.-amylases, selected from the group consisting of: (i) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 2 and (b) one or more variants as defined in claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6; (ii) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6; and (iii) a mixture of (a) one or more variants according claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6.

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L2: Entry 2 of 2 File: USPT Aug 20, 2002

US-PAT-NO: 6436888
DOCUMENT-IDENTIFIER: US 6436888 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd			DK

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Novozymes A/S	Bagsvaerd			DK	03

APPL-NO: 09/ 672459 [PALM]
DATE FILED: September 28, 2000

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of U.S. application Ser. No. 09/182,859 filed Oct. 29, 1998, now U.S. Pat. No. 6,143,708, which is a continuation of PCT/DK97/00197 filed Apr. 30, 1997 which claims priority under 35 U.S.C. 119 of Danish applications 0515/96 filed Apr. 30, 1996, 0712/96 filed Jun. 28, 1996, 0775/96 filed Jul. 11, 1996, and 1263/96 filed Nov. 8, 1996, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0515/96	April 30, 1996
DK	0712/96	June 28, 1996
DK	0775/96	July 11, 1996
DK	1263/96	November 8, 1996

INT-CL: [07] C12 N 9/28, C12 N 1/20, C12 N 15/00, C07 H 21/04

US-CL-ISSUED: 510/226; 435/202, 435/252.3, 435/320.1, 536/23.2, 536/23.7, 510/326, 510/392
US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

FIELD-OF-SEARCH: 510/226, 510/326, 510/392, 435/202, 435/252.3, 435/320.1, 536/23.2, 536/23.7

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL | Clear

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	5731280	March 1998	Nielsen et al.	510/392
☐	5736499	April 1998	Mitchinson et al.	510/392
☐	5824532	October 1998	Barnett et al.	435/202
☐	6143708	November 2000	Svendsen et al.	510/226

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 91/00353	January 1991	WO	
WO 95/10603	April 1995	WO	
WO 95/35382	December 1995	WO	
WO 96/23874	August 1996	WO	

ART-UNIT: 1652

PRIMARY-EXAMINER: Saldha; Tekchand

ATTY-AGENT-FIRM: Lambiris; Elias Garbell; Jason

ABSTRACT:

The invention relates to a variant of a parent Termamyl-like a-amylase, which variant has a-amylase activity and exhibits an alteration in at least one of the following properties relative to parent a-amylase: substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH/activity profile, pH/stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity.

16 Claims, 9 Drawing figures

First Hit Fwd Refs

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L3: Entry 1 of 5 File: USPT Jan 6, 2004

US-PAT-NO: 6673589
DOCUMENT-IDENTIFIER: US 6673589 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: January 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchert; Torben Vedel	Copenhagen .O slashed.			DK
Svendsen; Allan	Birker.o slashed.d			DK
Andersen; Carsten	Vaerloese			DK
Nielsen; Bjarne	Virum			DK
Nissen; Torben Lauesgaard	Frederiksberg C			DK
Kj.ae butted.rulff; S.o slashed.ren	Vanl.o slashed.se			DK

US-CL-CURRENT: 435/202; 510/226, 510/236, 510/320, 510/396

CLAIMS:

What is claimed is:

1. A variant of a parent Termamyl-like .alpha.-amylase, wherein said variant has .alpha.-amylase activity and at least 80% sequence identity to said parent .alpha.-amylase and comprises one or more mutations at a position corresponding to a position in the amino acid sequence shown in SEQ ID NO: 2 selected from the group consisting of: T461P; Q174*; R181Q,N,S; and G182T,S,N.

2. The variant according to claim 1, wherein the variant further has one or more of the following substitutions or deletions: K142R; S193P; N195F; K269R,Q, N270Y,R,D; K311R; E346Q; K385R; K458R; P459T; D183*; G184*; K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V; A186T,S,N,I,V,R; and W189T,S,N,Q.

3. The variant according to claim 1, wherein said variant exhibits improved stability at pH 8 to 10.5 as compared to said parent .alpha.-amylase.

4. The variant according to claim 1, wherein said variant exhibits improved Ca.sup.2+ stability at pH 8 to 10.5 as compared to said parent .alpha.-amylase.

5. The variant according to claim 1, wherein the parent Termamyl-like .alpha.-amylase is selected from the group consisting of: (i) Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in SEQ ID NO: 1; (ii) B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID NO: 5; and (iii) B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID

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Search Results - Record(s) 1 through 5 of 5 returned.

☐ 1. Document ID: US 6673589 B2

L3: Entry 1 of 5 File: USPT Jan 6, 2004

US-PAT-NO: 6673589
DOCUMENT-IDENTIFIER: US 6673589 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: January 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Borchert; Torben Vedel	Copenhagen .O slashed.			DK
Svendsen; Allan	Birker.o slashed.d			DK
Andersen; Carsten	Vaerloese			DK
Nielsen; Bjarne	Virum			DK
Nissen; Torben Lauesgaard	Frederiksberg C			DK
Kj.ae butted.rulff; S.o slashed.ren	Vanl.o slashed.se			DK

US-CL-CURRENT: 435/202; 510/226, 510/236, 510/320, 510/396

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 2. Document ID: US 6642044 B2

L3: Entry 2 of 5 File: USPT Nov 4, 2003

US-PAT-NO: 6642044
DOCUMENT-IDENTIFIER: US 6642044 B2

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisgard-Frantzen; Henrik	Bagsvaerd			DK

US-CL-CURRENT: 435/252.3; 435/202, 435/320.1, 510/226, 536/23.2, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 3. Document ID: US 6440716 B1

L3: Entry 3 of 5 File: USPT Aug 27, 2002

US-PAT-NO: 6440716
DOCUMENT-IDENTIFIER: US 6440716 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 27, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birkeroed			DK
Bisg.ang.rd-Frantzen; Henrik	Lyngby			DK
Borchert; Torben Vedel	Copenhagen			DK

US-CL-CURRENT: 435/202

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 4. Document ID: US 6436888 B1

L3: Entry 4 of 5 File: USPT Aug 20, 2002

US-PAT-NO: 6436888
DOCUMENT-IDENTIFIER: US 6436888 B1

TITLE: .alpha.-amylase mutants

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd			DK

US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 5. Document ID: US 6143708 A

L3: Entry 5 of 5 File: USPT Nov 7, 2000

US-PAT-NO: 6143708
DOCUMENT-IDENTIFIER: US 6143708 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd			DK

US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

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L3: Entry 5 of 5 File: USPT Nov 7, 2000

US-PAT-NO: 6143708
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TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 7, 2000

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US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

CLAIMS:

We claim:

1. A variant of a parent Termamyl-like .alpha.-amylase, wherein said variant has .alpha.-amylase activity and exhibits an alteration relative to said parent .alpha.-amylase in at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH-activity profile, pH-stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity; said variant comprising at least one mutation corresponding to a mutation in the amino acid sequence of SEQ ID NO:2 selected from the group consisting of:

a) single substitutions of A181E,D,Q,N,T or V; I201W,F, or L; Q9K,L, or E; F11R,K, or E; E12Q; D100N, or L; V101H,R,K,D,E, or F; I103H or K; N104R or K; H105R,K,D,E,W, or F; L196D,E,F, or Y; I212D, or E; L230H or K; A232H,F or V; V233D; K234L; I236N,H,D, or E; L241R,K,D,E, or F; A260S; W263H; Q264R,D,K,A,L,S,T or E; N265K,R, A,S,T or D; A269R,D, or E; L270R,K,H,D, or E; V283H, or D; F284H; D285N or L; V286R,K,H,D, or E; Y290R or K; V312R,K,D, or E; F323H; D325N; N326K,H,D, or L; H327Q,N,E,D, or F; Q330L, or E; G332D; Q333H,E, or L; S334A,V,T,L,I, or D; L335G,A,S,T, or N; R375E; T338D or E; Q360K,R, or E; D365N; G371D or R; H140Y; H142Y; H159Y; R169I,L,F, or T; R173I,L,F, or T; H156D; I212F; A209L,T; or V208I; N172R,H, or K; N188P; N190L or F; H205C; D207Y; E211Q;

b) multiple substitutions of H140D and H142R; H140K and H142D; H142Y and H156Y; Q264S and N265Y; H156Y and A181T and A209V; or H156Y and A181T and N190F and A209V and Q264S;

c) any substitution at positions R169, R173, H91, K389, R483, A181, H205, D207, or E211;

d) deletion of three amino acids within the sequence T369-I377;

e) deletions of D372, S373, and Q374;

f) replacement of T369-I377 with a sequence selected from the group consisting of I-P-T-H-S-V, I-P-T-H-G-V, and I-P-Q-Y-N-I;

g) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and A209V;

h) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V; and

i) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V and Q264S.

2. A variant according to claim 1 which comprises mutations selected from the group consisting of

substitution of H156Y and A181T and A209V;

substitution of H156Y and A181T and N190F and A209V and Q264S;

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and A209V;

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V; and

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V and Q264S.

3. A variant according to claim 1, wherein the parent Termamyl-like .alpha.-amylase is selected from the group consisting of:

the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID No. 2,

the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID No. 4,

the B. stearothermophilus .alpha.-amylase having the sequence shown in SEQ ID No. 6,

the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in FIGS. 1 and 2,

the Bacillus strain NCIB 12513 .alpha.-amylase having the sequence shown in FIG. 2, and

the Bacillus sp. #707 .alpha.-amylase having the sequence shown in FIG. 2.

4. A DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant according to claim 1.

5. A recombinant expression vector which carries a DNA construct according to claim 4.

6. A cell which is transformed a vector according to claim 5.

7. A cell according to claim 6, wherein said cell is a microorganism.

8. A cell according to claim 7, wherein said cell is a bacterium or a fungus.

9. The cell according to claim 8, wherein said cell is a gram positive bacterium selected from the group consisting of Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus and Bacillus thuringiensis.

10. A method for washing an object comprising contacting said object with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said washing.

11. A method for textile desizing comprising contacting said textile with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said desizing.

12. A method for starch liquefaction comprising contacting said starch with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said liquefaction.

13. A detergent additive comprising an .alpha.-amylase variant according to claim 1.

14. A detergent additive according to claim 13 which contains 0.02-200 mg of enzyme protein/g of the additive.

15. A detergent additive according to claim 13, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

16. A detergent composition comprising an .alpha.-amylase variant according to claim 1.

17. A detergent composition according to claim 16, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

18. A manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant according to claim 1.

19. A dishwashing detergent composition according to claim 18, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

20. A manual or automatic laundry washing composition comprising an .alpha.-amylase variant

according to claim 1.

21. A laundry washing composition according to claim 20, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, an amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

22. A composition comprising a mixture of .alpha.-amylases, selected from the group consisting of:

(i) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 2 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6;

(ii) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6; and

(iii) a mixture of (a) one or more variants according claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6.

23. A method for producing a variant of a parent Termamyl-like .alpha.-amylase of claim 1, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising:

(a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis,

(b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

(c) screening the host cells to identify a host cell expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

24. A polypeptide comprising

(a) a first peptide sequence consisting of residues 1-33 of SEQ ID NO:4 fused to

(b) a second peptide sequence consisting of a variant of residues 36-483 of SEQ ID NO:2, wherein said second peptide sequence has substitutions H156Y and A181T and N190F and A209V and Q264S relative to SEQ ID NO:2.

25. The polypeptide of claim 24, further comprising a substitution selected from the group consisting of: V54L,I,F,Y,W,R,K,H,E, and Q.

26. The variant of claim 2, further comprising a substitution selected from the group consisting of: V54L,I,F,Y,W,R,K,H,E, and Q.

27. A variant according to claim 1, wherein said mutation is I201W,F, or L.

28. A variant according to claim 1, wherein said mutation is Q9K,L, or E.

29. A variant according to claim 1, wherein said mutation is F11R,K, or E.

30. A variant according to claim 1, wherein said mutation is E12Q.

31. A variant according to claim 1, wherein said mutation is D100N, or L.

32. A variant according to claim 1, wherein said mutation is V101H,R,K,D,E or F.

33. A variant according to claim 1, wherein said mutation is I103H or K.

34. A variant according to claim 1, wherein said mutation is N104R or K.

35. A variant according to claim 1, wherein said mutation is H105R,K,D,E,W, or F.

36. A variant according to claim 1, wherein said mutation is L196D,E,F, or Y.

37. A variant according to claim 1, wherein said mutation is I212D or E.

38. A variant according to claim 1, wherein said mutation is L230H or K.

39. A variant according to claim 1, wherein said mutation is A232H,F or V.

40. A variant according to claim 1, wherein said mutation is V233D; K234L.

41. A variant according to claim 1, wherein said mutation is I236N,H,D, or E.

42. A variant according to claim 1, wherein said mutation is L241R,K,D,E, or F.

43. A variant according to claim 1, wherein said mutation is A260S.

44. A variant according to claim 1, wherein said mutation is W263H.

45. A variant according to claim 1, wherein said mutation is Q264R,D,K, A, L, S, T or E.

46. A variant according to claim 1, wherein said mutation is N265K,R, A,S,T or D.

47. A variant according to claim 1, wherein said mutation is A269R, D or E.

48. A variant according to claim 1, wherein said mutation is L270R,K,H,D, or E.

49. A variant according to claim 1, wherein said mutation is V283H, or D.

50. A variant according to claim 1, wherein said mutation is F284H.

51. A variant according to claim 1, wherein said mutation is D285N or L.

52. A variant according to claim 1, wherein said mutation is V286R,K,H,D or E.

53. A variant according to claim 1, wherein said mutation is Y290R or K.

54. A variant according to claim 1, wherein said mutation is V312R,K,D, or E.

55. A variant according to claim 1, wherein said mutation is F323H.

56. A variant according to claim 1, wherein said mutation is D325N.

57. A variant according to claim 1, wherein said mutation is N326K,H,D, or L.

58. A variant according to claim 1, wherein said mutation is H327Q,N,E,D, or F.

59. A variant according to claim 1, wherein said mutation is Q330L or E.

60. A variant according to claim 1, wherein said mutation is G332D.

61. A variant according to claim 1, wherein said mutation is Q333H,E or L.

62. A variant according to claim 1, wherein said mutation is S334A,V,T,L,I, or D.

63. A variant according to claim 1, wherein said mutation is L335G,A,S,T, or N.

64. A variant according to claim 1, wherein said mutation is R375E.

65. A variant according to claim 1, wherein said mutation is T338D or E.

66. A variant according to claim 1, wherein said mutation is Q360K,R, or E.

67. A variant according to claim 1, wherein said mutation is D365N.

68. A variant according to claim 1, wherein said mutation is G371D or R.

69. A variant according to claim 1, wherein said mutation is H140Y.

70. A variant according to claim 1, wherein said mutation is H142Y.

71. A variant according to claim 1, wherein said mutation is H159Y.

72. A variant according to claim 1, wherein said mutation is H156D.

73. A variant according to claim 1, wherein said mutation is I212F.

74. A variant according to claim 1, wherein said mutation is A209L,T; or V208I.

75. A variant according to claim 1, wherein said mutation is N172R,H, or K.

76. A variant according to claim 1, wherein said mutation is N188P.

77. A variant according to claim 1, wherein said mutation is N190L or F.

78. A variant according to claim 1, wherein said mutation is any substitution at position R169.

79. A variant according to claim 78, wherein said mutation is R169I,L,F, or T.

80. A variant according to claim 1, wherein said mutation is any substitution at position R173.

81. A variant according to claim 80, wherein said mutation is R173I,L,F, or T.

82. A variant according to claim 1, wherein said mutation is any substitution at position H91.

83. A variant according to claim 1, wherein said mutation is any substitution at position K389.

84. A variant according to claim 1, wherein said mutation is any substitution at position R483.

85. A variant according to claim 1, wherein said mutation is any substitution at position A181.

86. A variant according to claim 85, wherein said mutation is A181E,D,Q,N,T or V.

87. A variant according to claim 1, wherein said mutation is any substitution at position H205.

88. A variant according to claim 87, wherein said mutation is H205C.

89. A variant according to claim 1, wherein said mutation is any substitution at position D207.

90. A variant according to claim 89, wherein said mutation is D207Y.

91. A variant according to claim 1, wherein said mutation is any substitution at position E211.

92. A variant according to claim 91, wherein said mutation is E211Q.

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L3: Entry 5 of 5 File: USPT Nov 7, 2000

US-PAT-NO: 6143708
DOCUMENT-IDENTIFIER: US 6143708 A

TITLE: .alpha.-amylase mutants

DATE-ISSUED: November 7, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Svendsen; Allan	Birker.o slashed.d			DK
Borchert; Torben Vedel	Jyllinge			DK
Bisg.ang.rd-Frantzen; Henrik	Bagsv.ae butted.rd			DK

US-CL-CURRENT: 510/226; 435/202, 435/252.3, 435/320.1, 510/326, 510/392, 536/23.2, 536/23.7

CLAIMS:

We claim:

1. A variant of a parent Termamyl-like .alpha.-amylase, wherein said variant has .alpha.-amylase activity and exhibits an alteration relative to said parent .alpha.-amylase in at least one property selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, thermal stability, pH-activity profile, pH-stability profile, stability towards oxidation, Ca.sup.2+ dependency and specific activity; said variant comprising at least one mutation corresponding to a mutation in the amino acid sequence of SEQ ID NO:2 selected from the group consisting of:

a) single substitutions of A181E,D,Q,N,T or V; I201W,F, or L; Q9K,L, or E; F11R,K, or E; E12Q; D100N, or L; V101H,R,K,D,E, or F; I103H or K; N104R or K; H105R,K,D,E,W, or F; L196D,E,F, or Y; I212D, or E; L230H or K; A232H,F or V; V233D; K234L; I236N,H,D, or E; L241R,K,D,E, or F; A260S; W263H; Q264R,D,K,A,L,S,T or E; N265K,R, A,S,T or D; A269R,D, or E; L270R,K,H,D, or E; V283H, or D; F284H; D285N or L; V286R,K,H,D, or E; Y290R or K; V312R,K,D, or E; F323H; D325N; N326K,H,D, or L; H327Q,N,E,D, or F; Q330L, or E; G332D; Q333H,E, or L; S334A,V,T,L,I, or D; L335G,A,S,T, or N; R375E; T338D or E; Q360K,R, or E; D365N; G371D or R; H140Y; H142Y; H159Y; R169I,L,F, or T; R173I,L,F, or T; H156D; I212F; A209L,T; or V208I; N172R,H, or K; N188P; N190L or F; H205C; D207Y; E211Q;

b) multiple substitutions of H140D and H142R; H140K and H142D; H142Y and H156Y; Q264S and N265Y; H156Y and A181T and A209V; or H156Y and A181T and N190F and A209V and Q264S;

c) any substitution at positions R169, R173, H91, K389, R483, A181, H205, D207, or E211;

d) deletion of three amino acids within the sequence T369-I377;

e) deletions of D372, S373, and Q374;

f) replacement of T369-I377 with a sequence selected from the group consisting of I-P-T-H-S-V, I-P-T-H-G-V, and I-P-Q-Y-N-I;

g) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and A209V;

h) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V; and

i) deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V and Q264S.

2. A variant according to claim 1 which comprises mutations selected from the group consisting of

substitution of H156Y and A181T and A209V;

substitution of H156Y and A181T and N190F and A209V and Q264S;

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and A209V;

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V; and

deletion of A1 and N2 and substitution of L3V and M15T and R23K and S29A and A30E and Y31H and A33S and E34D and H35I and H156Y and A181T and N190F and A209V and Q264S.

3. A variant according to claim 1, wherein the parent Termamyl-like .alpha.-amylase is selected from the group consisting of:

the B. licheniformis .alpha.-amylase having the sequence shown in SEQ ID No. 2,

the B. amyloliquefaciens .alpha.-amylase having the sequence shown in SEQ ID No. 4,

the B. stearothermophilus .alpha.-amylase having the sequence shown in SEQ ID No. 6,

the Bacillus strain NCIB 12512 .alpha.-amylase having the sequence shown in FIGS. 1 and 2,

the Bacillus strain NCIB 12513 .alpha.-amylase having the sequence shown in FIG. 2, and

the Bacillus sp. #707 .alpha.-amylase having the sequence shown in FIG. 2.

4. A DNA construct comprising a DNA sequence encoding an .alpha.-amylase variant according to claim 1.

5. A recombinant expression vector which carries a DNA construct according to claim 4.

6. A cell which is transformed a vector according to claim 5.

7. A cell according to claim 6, wherein said cell is a microorganism.

8. A cell according to claim 7, wherein said cell is a bacterium or a fungus.

9. The cell according to claim 8, wherein said cell is a gram positive bacterium selected from the group consisting of Bacillus subtilis, Bacillus licheniformis, Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus, Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus and Bacillus thuringiensis.

10. A method for washing an object comprising contacting said object with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said washing.

11. A method for textile desizing comprising contacting said textile with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said desizing.

12. A method for starch liquefaction comprising contacting said starch with an .alpha.-amylase variant according to claim 1 under conditions sufficient for said liquefaction.

13. A detergent additive comprising an .alpha.-amylase variant according to claim 1.

14. A detergent additive according to claim 13 which contains 0.02-200 mg of enzyme protein/g of the additive.

15. A detergent additive according to claim 13, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

16. A detergent composition comprising an .alpha.-amylase variant according to claim 1.

17. A detergent composition according to claim 16, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

18. A manual or automatic dishwashing detergent composition comprising an .alpha.-amylase variant according to claim 1.

19. A dishwashing detergent composition according to claim 18, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, another amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

20. A manual or automatic laundry washing composition comprising an .alpha.-amylase variant

according to claim 1.

21. A laundry washing composition according to claim 20, further comprising a second enzyme selected from the group consisting of a protease, a lipase, a peroxidase, an amylolytic enzyme, a cellulase, and combinations of any of the foregoing.

22. A composition comprising a mixture of .alpha.-amylases, selected from the group consisting of:

(i) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 2 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6;

(ii) a mixture of (a) a polypeptide having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6; and

(iii) a mixture of (a) one or more variants according claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase having the sequence shown in SEQ ID No. 6 and (b) one or more variants according to claim 1, wherein said variants are derived from a parent Termamyl-like .alpha.-amylase other than SEQ ID NO:6.

23. A method for producing a variant of a parent Termamyl-like .alpha.-amylase of claim 1, which variant exhibits increased stability at low pH and at low calcium concentration relative to the parent, the method comprising:

(a) subjecting a DNA sequence encoding the parent Termamyl-like .alpha.-amylase to random mutagenesis,

(b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

(c) screening the host cells to identify a host cell expressing a mutated .alpha.-amylase which has increased stability at low pH and low calcium concentration relative to the parent .alpha.-amylase.

24. A polypeptide comprising

(a) a first peptide sequence consisting of residues 1-33 of SEQ ID NO:4 fused to

(b) a second peptide sequence consisting of a variant of residues 36-483 of SEQ ID NO:2, wherein said second peptide sequence has substitutions H156Y and A181T and N190F and A209V and Q264S relative to SEQ ID NO:2.

25. The polypeptide of claim 24, further comprising a substitution selected from the group consisting of: V54L,I,F,Y,W,R,K,H,E, and Q.

26. The variant of claim 2, further comprising a substitution selected from the group consisting of: V54L,I,F,Y,W,R,K,H,E, and Q.

27. A variant according to claim 1, wherein said mutation is I201W,F, or L.

28. A variant according to claim 1, wherein said mutation is Q9K,L, or E.

29. A variant according to claim 1, wherein said mutation is F11R,K, or E.

30. A variant according to claim 1, wherein said mutation is E12Q.

31. A variant according to claim 1, wherein said mutation is D100N, or L.

32. A variant according to claim 1, wherein said mutation is V101H,R,K,D,E or F.

33. A variant according to claim 1, wherein said mutation is I103H or K.

34. A variant according to claim 1, wherein said mutation is N104R or K.

35. A variant according to claim 1, wherein said mutation is H105R,K,D,E,W, or F.

36. A variant according to claim 1, wherein said mutation is L196D,E,F, or Y.

37. A variant according to claim 1, wherein said mutation is I212D or E.

38. A variant according to claim 1, wherein said mutation is L230H or K.

39. A variant according to claim 1, wherein said mutation is A232H,F or V.

40. A variant according to claim 1, wherein said mutation is V233D; K234L.

41. A variant according to claim 1, wherein said mutation is I236N,H,D, or E.

42. A variant according to claim 1, wherein said mutation is L241R,K,D,E, or F.

43. A variant according to claim 1, wherein said mutation is A260S.

44. A variant according to claim 1, wherein said mutation is W263H.

45. A variant according to claim 1, wherein said mutation is Q264R,D,K, A, L, S, T or E.

46. A variant according to claim 1, wherein said mutation is N265K,R, A,S,T or D.

47. A variant according to claim 1, wherein said mutation is A269R, D or E.

48. A variant according to claim 1, wherein said mutation is L270R,K,H,D, or E.

49. A variant according to claim 1, wherein said mutation is V283H, or D.

50. A variant according to claim 1, wherein said mutation is F284H.

51. A variant according to claim 1, wherein said mutation is D285N or L.

52. A variant according to claim 1, wherein said mutation is V286R,K,H,D or E.

53. A variant according to claim 1, wherein said mutation is Y290R or K.

54. A variant according to claim 1, wherein said mutation is V312R,K,D, or E.

55. A variant according to claim 1, wherein said mutation is F323H.

56. A variant according to claim 1, wherein said mutation is D325N.

57. A variant according to claim 1, wherein said mutation is N326K,H,D, or L.

58. A variant according to claim 1, wherein said mutation is H327Q,N,E,D, or F.

59. A variant according to claim 1, wherein said mutation is Q330L or E.

60. A variant according to claim 1, wherein said mutation is G332D.

61. A variant according to claim 1, wherein said mutation is Q333H,E or L.

62. A variant according to claim 1, wherein said mutation is S334A,V,T,L,I, or D.

63. A variant according to claim 1, wherein said mutation is L335G,A,S,T, or N.

64. A variant according to claim 1, wherein said mutation is R375E.

65. A variant according to claim 1, wherein said mutation is T338D or E.

66. A variant according to claim 1, wherein said mutation is Q360K,R, or E.

67. A variant according to claim 1, wherein said mutation is D365N.

68. A variant according to claim 1, wherein said mutation is G371D or R.

69. A variant according to claim 1, wherein said mutation is H140Y.

70. A variant according to claim 1, wherein said mutation is H142Y.

71. A variant according to claim 1, wherein said mutation is H159Y.

72. A variant according to claim 1, wherein said mutation is H156D.

73. A variant according to claim 1, wherein said mutation is I212F.

74. A variant according to claim 1, wherein said mutation is A209L,T; or V208I.

75. A variant according to claim 1, wherein said mutation is N172R,H, or K.

76. A variant according to claim 1, wherein said mutation is N188P.

77. A variant according to claim 1, wherein said mutation is N190L or F.

78. A variant according to claim 1, wherein said mutation is any substitution at position R169.

79. A variant according to claim 78, wherein said mutation is R169I,L,F, or T.

80. A variant according to claim 1, wherein said mutation is any substitution at position R173.

81. A variant according to claim 80, wherein said mutation is R173I,L,F, or T.

82. A variant according to claim 1, wherein said mutation is any substitution at position H91.

83. A variant according to claim 1, wherein said mutation is any substitution at position K389.

84. A variant according to claim 1, wherein said mutation is any substitution at position R483.

85. A variant according to claim 1, wherein said mutation is any substitution at position A181.

86. A variant according to claim 85, wherein said mutation is A181E,D,Q,N,T or V.

87. A variant according to claim 1, wherein said mutation is any substitution at position H205.

88. A variant according to claim 87, wherein said mutation is H205C.

89. A variant according to claim 1, wherein said mutation is any substitution at position D207.

90. A variant according to claim 89, wherein said mutation is D207Y.

91. A variant according to claim 1, wherein said mutation is any substitution at position E211.

92. A variant according to claim 91, wherein said mutation is E211Q.

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Tuesday, May 11, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB; PLUR=YES; OP=ADJ		
☐	L10	L7 with 405	1
	DB=USPT; PLUR=YES; OP=ADJ		
☐	L9	L7 with 405	0
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L8	L7 with 405	1
☐	L7	amylase with variant.clm.	57
☐	L6	amylase with variant?	492
☐	L5	alpha.-amylase with variant?	0
☐	L4	alpha.-amylase with mutant?	0
	DB=USPT; PLUR=YES; OP=ADJ		
☐	L3	6,143,708	5
☐	L2	6,436,888	2
☐	L1	6,642,044	1

END OF SEARCH HISTORY

5989169 A

Detailed Description Text (101):
Accordingly, the variant according to this aspect of the invention is preferably one, which has been modified in one or more amino acid residues present within 10 .ANG. from a calcium and/or sodium ion identified in the three-dimensional Termamyl-like .alpha.-amylase structure in such a manner that the affinity of the .alpha.-amylase for calcium is increased.

Detailed Description Text (103):
V102, I103, N104, H105, K106, R125, W155, W157, Y158, H159, F160, D161, G162, T163, Y175, K176, F177, G178, K180, A181, W182, D183, W184, E185, V186, S187, N192, Y193, D194, Y195, L196, M197, Y198, A199, D200, I201, D202, Y203, D204, H205, P206, V208, A209, D231, A232, V233, K234, H235, I236, K237, F238, F240, L241, A294, A295, S296, T297, Q298, G299, G300, G301, Y302, D303, M304, R305, K306, L307, W342, F343, L346, Q393, Y394, Y396, H405, H406, D407, I408, V409, R413, E414, G415, D416, S417, V419, A420, N421, S422, G423, L424, I428, T429, D430, G431, P432, V440, G441, R442, Q443, N444, A445, G446, E447, T448, W449, I462, G475, Y480, V481, Q482, R483.

Detailed Description Text (104):
In order to construct a variant according to this aspect of the invention it is desirable to replace at least one of the above mentioned amino acid residues (or an amino acid residue occupying an equivalent position in another Termamyl-like .alpha.-amylase than that defined by SEQ ID NO 2), which is contemplated to be involved in providing a non-optimal calcium binding, with any other amino acid residue which improves the Ca.sup.2+ binding affinity of the variant enzyme. In practice, the identification and subsequent modification of the amino acid residue is performed by the following method:

WEST Search History

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DATE: Tuesday, May 11, 2004

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	DB=USPT; PLUR=YES; OP=ADJ		
☐	L5	termamyl-like and H405	6
☐	L4	termamyl-like and 405	13
☐	L3	termamyl-like with 405	0
☐	L2	L1 and 405	5
☐	L1	termamyl-like.clm.	8

END OF SEARCH HISTORY